

# Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/135850/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Waite, Adrian J., Millar, David and Clarke, Angus ORCID:  
<https://orcid.org/0000-0002-1200-9286> 2020. The generation of an induced pluripotent stem cell line (DCGi001-A) from an individual with FOXG1 syndrome carrying the c.460dupG (p.Glu154fs) variation in the FOXG1 gene. Stem Cell Research 49 , 102018. 10.1016/j.scr.2020.102018 file

Publishers page: <http://dx.doi.org/10.1016/j.scr.2020.102018>  
<<http://dx.doi.org/10.1016/j.scr.2020.102018>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies.

See

<http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.





Lab resource: Stem Cell Line

# The generation of an induced pluripotent stem cell line (DCGi001-A) from an individual with FOXG1 syndrome carrying the c.460dupG (p.Glu154fs) variation in the *FOXG1* gene

Adrian J. Waite, David Millar, Angus Clarke\*

Division of Cancer and Genetics, Cardiff University School of Medicine, Institute of Medical Genetics Building, Heath Park, Cardiff, Wales CF14 4XN, United Kingdom

## ABSTRACT

FOXG1 syndrome is a neurodevelopmental disorder caused by mutations in the *FOXG1* gene. Here, an induced pluripotent stem cell (iPSC) line was generated from human dermal fibroblasts of an individual with the c.490dupG (p.Glu154fs) mutation in the *FOXG1* gene. Fibroblasts were reprogrammed using non-integrating episomal plasmids and pluripotency marker expression was confirmed by both immunocytochemistry and quantitative PCR in the resultant iPSC line. There were no karyotypic abnormalities and the cell line successfully differentiated into all three germ layers. This cell line may prove useful in the study of the pathogenic mechanisms that underpin FOXG1 syndrome.

## 1. Resource Table

Unique stem cell line identifier	DCGi001-A
Alternative names of stem cell line	FS_E154#3
Institution	Division of Cancer and Genetics, Cardiff University School of Medicine, UK
Contact information of distributor	Professor Angus Clarke, <a href="mailto:ClarkeAJ@cardiff.ac.uk">ClarkeAJ@cardiff.ac.uk</a> or Professor Andrew Tee, <a href="mailto:teea@cardiff.ac.uk">teea@cardiff.ac.uk</a>
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 18 Sex: Female Ethnicity: White European
Cell Source	Human dermal fibroblasts (HDF)
Clonality	Clonal
Method of reprogramming	Episomal plasmid-based iPSC reprogramming
Genetic modification	YES
Type of modification	Spontaneous heterozygous mutation NM_005249.5:c.460dup (p.Glu154fs)
Associated disease	FOXG1 Syndrome (OMIM # 613454)
Gene/locus	<i>FOXG1</i> /14q12
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	06–2019
Cell line repository/bank	<a href="https://hpscreg.eu/cell-line/DCGi001-A">https://hpscreg.eu/cell-line/DCGi001-A</a>
Ethical approval	South East Wales NHS Research Ethics Committee 10/WSE03/3

## 2. Resource utility

FOXG1 syndrome is a neurodevelopmental disorder caused by mutations in the *FOXG1* gene. We generated iPSCs from fibroblasts of an individual with the c.460dupG *FOXG1* mutation. This patient-derived cell line will be useful for modelling the pathogenic mechanisms that underpin FOXG1 syndrome.

## 3. Resource details

FOXG1 syndrome (originally called the congenital-onset variant of Rett syndrome) is a rare neurodevelopmental disorder associated with heterozygous mutations in the *FOXG1* gene (located on chromosome 14q12). The range of variants associated with the disorder include chromosomal microdeletions, larger deletions and intragenic mutations (missense, nonsense and frameshift). Furthermore, a subset of cases are associated with structural variants occurring downstream of *FOXG1* that may disrupt *cis*-regulation of *FOXG1* expression. The clinical phenotype associated with FOXG1 syndrome includes severe intellectual disability, postnatal microcephaly, dyskinetic-hyperkinetic movement disorders, visual impairment, epilepsy, stereotypies, abnormal sleep patterns, and unexplained episodes of crying. Brain imaging of patients reveal structural abnormalities including hypoplasia of the corpus callosum and underdevelopment of the frontal cortex (Kortum et al., 2011; Vegas et al., 2018). The *FOXG1* gene encodes the forkhead box G1 protein, a winged-helix transcriptional factor that is a master regulator of the development and regional specification of the ventral

\* Corresponding author.

E-mail address: [ClarkeAJ@cardiff.ac.uk](mailto:ClarkeAJ@cardiff.ac.uk) (A. Clarke).

<https://doi.org/10.1016/j.scr.2020.102018>

Received 29 January 2020; Received in revised form 21 September 2020; Accepted 27 September 2020

Available online 01 October 2020

1873-5061/ © 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

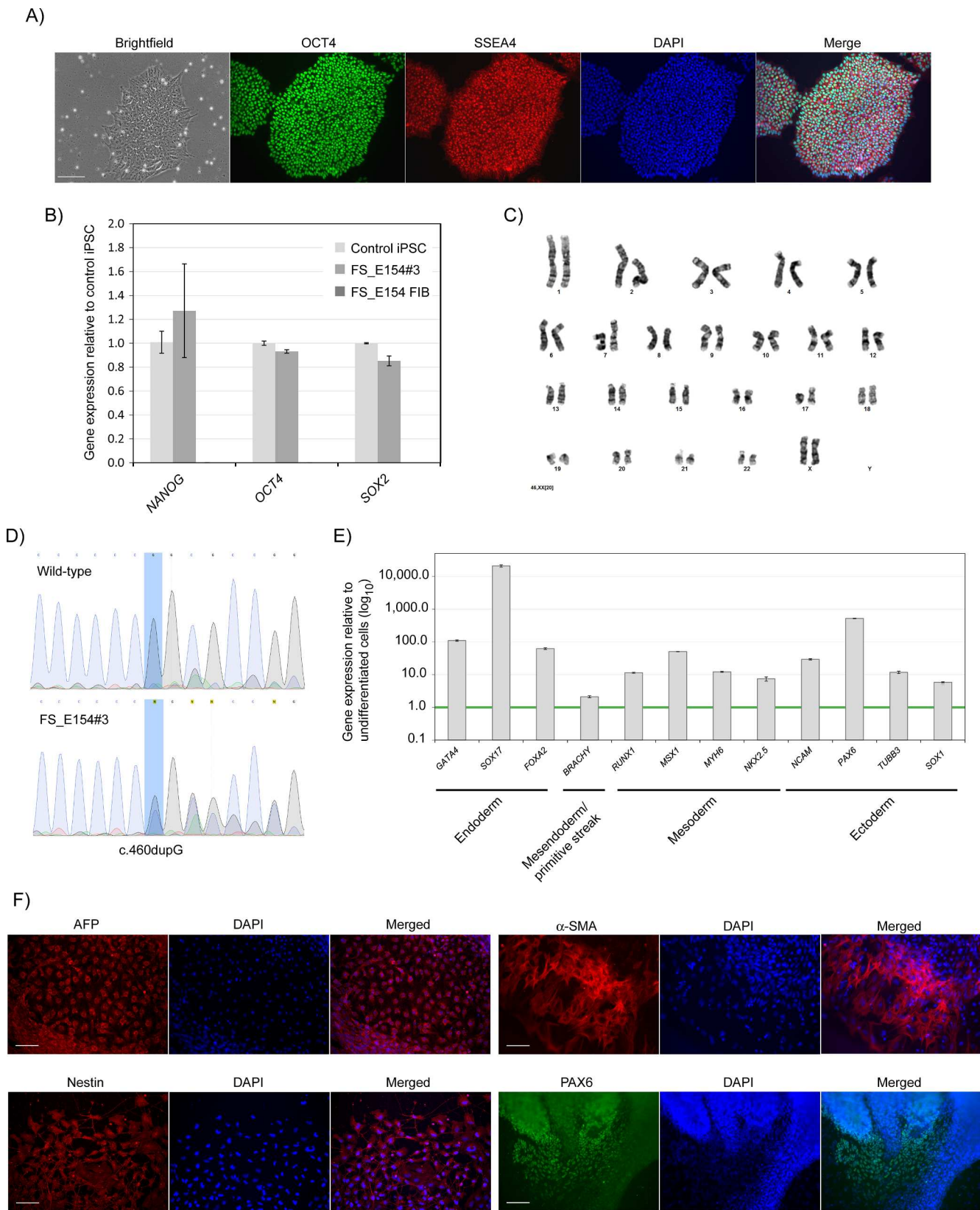


Fig. 1. Figure content described in Resource Details.

telencephalon (Wong et al., 2019). Through its array of protein and DNA interactions FOXG1 has pleiotropic and non-redundant roles in brain development, reflected in the complex genotype-phenotype presentations observed (Vegas et al., 2018; Mitter et al., 2018).

Fibroblasts were isolated from a skin biopsy taken from an 18-year old FOXG1 syndrome patient with a previously reported heterozygous pathogenic variant, c.460dupG (p.Glu154fs) (Vegas et al., 2018). iPSCs were generated by a non-integrating episomal plasmid-based method



**Table 1**  
Characterization and validation.

Classification	Test	Result	Data
Morphology	Brightfield microscopy	Normal hESC-like morphology	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Expression of OCT4 and SSEA4.	Fig. 1 panel A
Genotype	Quantitative analysis (RT-qPCR)	Expression of OCT4, SOX2 and NANOG.	Fig. 1 panel B
Identity	Karyotype (G-banding), passage 9	46XX, Resolution ( $\approx 5\text{--}10\text{ Mb}/\approx 450\text{-band}$ )	Fig. 1 panel C
	Microsatellite PCR (mPCR) OR	Not performed	
	STR analysis	16 loci matched	Available with the authors
Mutation analysis	Sequencing	Heterozygous FOXG1 c.460dupG	Fig. 1 panel D
	Southern Blot OR WGS	Not performed	
Microbiology and virology	Mycoplasma, tested by PCR-assay	Negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation	Expression of germ layer markers (qPCR): endoderm (GATA4, SOX17, FOXA2), mesoderm (RUNX1, MSX1, MYH6, NKX2.5), ectoderm (NCAM, PAX6, TUBB3, SOX1). Immunoreactivity for alpha-fetoprotein (endoderm), alpha smooth muscle actin (mesoderm), nestin (ectoderm) and paired box protein Pax-6 (ectoderm) by immunocytochemistry.	Fig. 1 panel E and F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	Not done
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	Not done
	HLA tissue typing	Not performed	Not done

through expression of the reprogramming factors OCT4, SOX2, KLF4, L-MYC, LIN28 and p53 shRNA. A panel of iPSC-like colonies were manually picked after 20 days post-electroporation and expanded in culture over several passages for establishment as iPSC clones for further characterisation. Amongst the panel of clones was FS\_E154#3 that is described herein. Polymerase chain reaction (PCR) analysis performed at the 10th passage using validated primers against the EBNA1 backbone, common to all the episomal plasmids, confirmed their elimination from the iPSC line (Supplementary Fig. 1A). The established FS\_E154#3 iPSC line showed typical human embryonic stem cell-like morphology as judged by brightfield microscopy (Fig. 1A, scale bar is 100  $\mu\text{m}$ ). The line expressed the pluripotency markers OCT4 and SSEA4 as shown by immunocytochemistry (Fig. 1A). Quantitative real-time PCR analysis (qPCR) demonstrated the expression of pluripotency markers NANOG, OCT4 and SOX2 in the FS\_E154#3 line comparable to a control iPSC line (sex-matched) previously generated in house (Fig. 1B). FS\_E154#3 displayed a normal diploid 46 XX karyotype (passage 9, Fig. 1C), and sequencing analysis confirmed the presence of the heterozygous FOXG1 variant found in the parental fibroblast line (Fig. 1D). Short tandem repeat analysis of genomic DNA extracted from FS\_E154#3 and parental fibroblasts confirmed matching genetic identity at the 16 loci tested. *in vitro* differentiation potential was verified using an embryoid body (EB) assay, followed by qPCR analysis of gene expression relative to undifferentiated cells (Fig. 1E, 12 germ layer markers). This detected increased expression of marker genes indicating the presence of cells derived from endoderm, ectoderm and mesoderm lineages. Furthermore, epifluorescence microscopy detected cells immunoreactive for ectoderm (nestin, PAX6), endoderm (AFP) and mesoderm ( $\alpha\text{SMA}$ ) specific markers (Fig. 1F). The absence of mycoplasma contamination was confirmed by a PCR-based assay (Supplementary Fig. 1B).

#### 4. Materials and methods

##### 4.1. Reagents

Reagents were purchased from Thermo Fisher Scientific unless otherwise stated.

##### 4.2. iPSC reprogramming and culture

iPSC derivation was achieved by episomal reprogramming using

plasmids pCXLE-hUL, pCXLE-hSK and pCXLE-hOCT3/4-shp53-F (Addgene; Supplementary Fig. 1C). Patient-derived fibroblasts ( $5 \times 10^5$  cells) were electroporated with all three vectors (1  $\mu\text{g}$  of each) using the Amara Human Dermal Fibroblast Nucleofector Kit and Nucleofector 2b (Lonza) on program U023. Nucleofected fibroblasts were plated onto Matrigel-coated dishes with culture conditions and iPSC colony isolation according to the Essential 6 (E6) media instructions (MAN0007568). iPSCs were propagated in StemFlex medium and generally passaged at a 1:6 ratio every 4 days using ReLeSR (STEMCELL Technologies).

##### 4.3. *In vitro* differentiation

EBs were formed as previously described (Ungrin et al., 2008) with modifications. iPSCs were dissociated using Accutase, seeded into v-bottom plates (Greiner; 2000 cells/well) and cultured for 24 h in StemFlex medium with 10  $\mu\text{M}$  Y-27632 (Hello Bio) and 0.4% (w/v) PVA (Sigma). EBs were washed in D-PBS, resuspended in E6 media and transferred to low-attachment 96-well plates (Greiner). EBs were cultured for a further 7 days before seeding onto Matrigel-coated 24-well plates and cultured in E6 media for an additional 7 days prior to analysis.

##### 4.4. RNA isolation, PCR and qPCR

RNA was isolated using the RNeasy Mini Kit (QIAGEN) followed by DNase treatment (DNasefree kit). Complementary DNA was reverse transcribed from 250 ng total RNA using SuperScript IV. qPCRs were performed using Power SYBR Green PCR master mix and an ABI 7300 Real Time PCR System, with data analysed by the  $\Delta\Delta\text{Ct}$  method using ACTNB for normalisation. PCR and qPCR parameters are available on request and Table 1.

##### 4.5. Immunocytochemistry

Cells were fixed in 4% paraformaldehyde for 20 mins at room temperature (RT), permeabilised in 0.1% (v/v) Triton X-100 for 15 mins at 4  $^{\circ}\text{C}$  and incubated in blocking solution (10% FBS in PBS) for 30 mins at RT. Fixed cells were incubated with primary antibodies (Table 2) in blocking solution (1–3 h at RT). Following three PBS washes, cells were incubated with Alexa Fluor-conjugated secondary antibodies (Table 2) in blocking solution (1 h at RT), washed three times

**Table 2**  
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:500	Abcam Cat# Ab19857, RRID:AB_445175
	Mouse anti-SSEA4	1:50	DSHB Cat# MC_813-70, RRID:AB_528477
Differentiation Markers	Mouse anti-AFP	1:20	R and D Systems Cat# MAB1368-SP, RRID:AB_357658
	Mouse anti-αSMA	1:50	R and D Systems Cat# MAB1420-SP, RRID:AB_262054
	Mouse anti-Nestin	1:50	R and D Systems Cat# MAB1259-SP, RRID:AB_2251304
	Rabbit anti-PAX6	1:100	Proteintech Cat# 12323-1-AP, RRID: AB_2159695
Secondary antibodies	Alexa Fluor 488 Donkey anti-Rabbit IgG	1:1500	Thermo Fisher Scientific Cat# A-21206, RRID:AB_2535792
	Alexa Fluor 568 Goat anti-Mouse IgG	1:1500	Thermo Fisher Scientific Cat# A-11031, RRID:AB_144696
Primers			
	Target	Forward/Reverse primer (5'-3')	
Episomal Plasmids (PCR)	<i>EBNA (all plasmids), expected product 61 bp</i>	ATCAGGGCCAAGACATAGAGATG/GCCAAATGCAACTGGACGTT	
	<i>NANOG</i>	TGAACCTCAGCTACAAACAG/TGGTGGTAGGAAGAGTAAAG	
Pluripotency Markers (qPCR)	<i>OCT4</i>	CCCCAGGGCCCCATTTTGGTACC/ACCTCAGTTTGAATGCATGGGAGAGC	
	<i>SOX2</i>	GCTTAGCCTCGTCGATGAAC/AACCCCAAGATGCACAACTC	
	<i>GATA4</i>	CTAGACCGTGGGTTTTCGAT/TGGGTAAAGTGCCCTGTAG	
	<i>SOX17</i>	CTCTGCCTCCTCCACGAA/CAGAATCCAGACCTGCACAA	
	<i>FOXA2</i>	GGAGCAGCTACTATGCAGAGC/CGTGTTCATGCCGTTTCATCC	
	<i>Brachyury</i>	TGAAGTGGGTCTCAGGGAAGCA/CCTTCAGCAAAGTCAAGCTCACC	
	<i>RUNX1</i>	CCCTAGGGGATGTTCCAGAT/TGAAGCTTTTCCCTCTTCCA	
	<i>MSX1</i>	CGAGAGGACCCCGTGGATGCAGAG/GGCGGCCATCTTCAGCTTCTCCAG	
	<i>MYH6</i>	TCAGCTGGAGGCCAAAGTAAAGGA/TTCTTGAGCTCTGAGCACTCGTCT	
	<i>NKX2.5</i>	CTAAACCTGGAACAGCAGCA/CGTAGGCCTCTGGCTTGA	
	<i>NCAM</i>	ATGGAACCTCTATTAAGTGAACCTG/TAGACCTCATACTCAGCATTCCAGT	
	<i>PAX6</i>	GTCCATCTTTGCTTGGGAAA/TAGCCAGGTTGCGAAGAAGT	
	<i>TUBB3</i>	CCCAGTATGAGGGAGATCGT/CGATGCCATGCTCATCAC	
	<i>SOX1</i>	ATTATTTTGCCCGTTTTCCT/CAAGGAAACACAATCGCTG	
House-Keeping Gene (qPCR)	<i>ACTNB</i>	TGCCGACAGGATGCAGAAAG/AGCGAGGCCAGGATGGA	
Genotyping (Pathogenic variant sequencing)	<i>FOXG1, expected product 894 bp</i>	ATCCCAAGTCTCGTTTCAG/CATGGGCCAGTAGAGGGAG	

with PBS and mounted in Antifading Mounting Medium containing DAPI counterstain (Dianova). Images were acquired using a Leica DM IL LED Microscope with Leica DMC3000 G CCD camera.

#### 4.6. Sequencing

The *FOXG1* c.460dupG variant was confirmed in iPSCs and parental fibroblasts by Sanger Sequencing of PCR-amplified sequence from genomic DNA using specific primers (Table 2). DNA was isolated using the QIAamp DNA mini kit (QIAGEN). Sequencing reactions were performed using Big Dye v3.1 chemistry and analysed by the All Wales Medical Genomics Service (Cardiff, UK).

#### 4.7. STR analysis

STR analysis of 16 loci was performed using the Promega Powerplex 16HS kit with amplicon detection using an Applied Biosystems 3730xl (undertaken by Source BioScience, Nottingham, UK).

#### 4.8. Karyotyping

G-banding analysis of 20 metaphase spreads was performed by Cell Guidance Systems (Cambridge, UK). Fixed iPSCs for analysis were prepared according to service provider instructions ([http://cellgs.e2ecd.co.uk/Downloads/Karyotype\\_FixedSamples.pdf](http://cellgs.e2ecd.co.uk/Downloads/Karyotype_FixedSamples.pdf)).

#### 4.9. Mycoplasma test

Mycoplasma contamination was tested using the PCR-based Venor®GeM Classic detection kit (Minerva Biolabs) according to manufacturer's instructions.

#### Acknowledgements

The authors are indebted to the patient who donated tissue for research. We are grateful to Dr Mouhamed Alsaqati and Professor Adrian Harwood (Neuroscience and Mental Health Research Institute, Cardiff University) for assistance with iPSC imaging. We thank Dr Alis Hughes for helpful discussion and critical reading of the manuscript. We thank the All Wales Medical Genomics Service (Institute of Medical Genomics, Cardiff, UK) for technical assistance. This project was supported by funding from the Blackswan Foundation.

#### Appendix A. Supplementary data

Supplementary Fig. 1 can be found online at <https://doi.org/10.1016/j.scr.2020.102018>.

#### References

- Kortum, F., Das, S., Flindt, M., Morris-Rosendahl, D.J., Stefanova, I., Goldstein, A., Horn, D., Klopocki, E., Kluger, G., Martin, P., Rauch, A., Roumer, A., Saitta, S., Walsh, L.E., Wiczorek, D., Uyanik, G., Kutsche, K., Dobyns, W.B., 2011. The core FOXG1 syndrome phenotype consists of postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum hypogenesis. *J. Med. Genet.* 48, 396–406.
- Mitter, D., Pringsheim, M., Kaulisch, M., Plumacher, K.S., Schroder, S., Warthemann, R., Abou Jamra, R., Baethmann, M., Bast, T., Buttel, H.M., Cohen, J.S., Conover, E., Courage, C., Eger, A., Fatemi, A., Grebe, T.A., Hauser, N.S., Heinritz, W., Helbig, K.L., Heruth, M., Huhle, D., Hoft, K., Karch, S., Kluger, G., Korenke, G.C., Lemke, J.R., Lutz, R.E., Patzer, S., Prehl, I., Hoertnagel, K., Ramsey, K., Rating, T., Riess, A., Rohena, L., Schimmel, M., Westman, R., Zech, F.M., Zoll, B., Malzahn, D., Zirn, B., Brockmann, K., 2018. FOXG1 syndrome: genotype-phenotype association in 83 patients with FOXG1 variants. *Genet. Med.* 20, 98–108.
- Ungrin, M.D., Joshi, C., Nica, A., Bauwens, C., Zandstra, P.W., 2008. Reproducible, ultra high-throughput formation of multicellular organization from single cell suspension-derived human embryonic stem cell aggregates. *PLoS One* 3, e1565.
- Vegas, N., Cavallin, M., Maillard, C., Boddaert, N., Toulouse, J., Schaefer, E., Lerman-

- Sagie, T., Lev, D., Magalie, B., Moutton, S., Haan, E., Isidor, B., Heron, D., Milh, M., Rondeau, S., Michot, C., Valence, S., Wagner, S., Hully, M., Mignot, C., Masurel, A., Datta, A., Odent, S., Nizon, M., Lazaro, L., Vincent, M., Cogne, B., Guerrot, A.M., Arpin, S., Pedespan, J.M., Caubel, I., Pontier, B., Troude, B., Rivier, F., Philippe, C., Bienvenu, T., Spitz, M.A., Bery, A., Bahi-Buisson, N., 2018. Delineating FOXG1 syndrome: From congenital microcephaly to hyperkinetic encephalopathy. *Neurol. Genet* 4, e281.
- Wong, L.C., Singh, S., Wang, H.P., Hsu, C.J., Hu, S.C., Lee, W.T., 2019. FOXG1-related syndrome: from clinical to molecular genetics and pathogenic mechanisms. *Int. J. Mol. Sci.* 20.